CRADA Opportunity: New Method for Detection of Highly Pathogenic *Salmonella*

Salmonella enterica is a leading agent of gastrointestinal illness in the US and worldwide. *Salmonella* are a diverse group of bacteria with over 1,700 serotypes noted for causing human illness. But while any *Salmonella* may make the right host sick when present at the right dose, only 15 serotypes are responsible for over two-thirds of documented illnesses in the U.S. each year. These top ranked *Salmonella* are able to infect more people, in part because of their virulence gene repertoire and a resultant decreased minimal infective dose.

US Meat Animal Research Center scientists conducted comparative genomic analyses of a variety of *Salmonella* strains associated with humans and cattle. These analyses revealed molecular targets for identifying four of the leading disease-causing *Salmonella* serotypes (Enteritidis, Typhimurium, (1,4,[5],12:i:-), and Newport) and a noted invasive serotype, *S.* Dublin. **These data were used to design a molecular assay targeting markers that are shared among serotypes noted for being invasive and/or causing the most human illnesses (i.e. Highly Pathogenic *Salmonella* or HPS) but are notably absent among serotypes with a lower frequency of association with human illness (Table 1).**

### Multiplex PCR Assay to identify **H**ighly **P**athogenic **S**almonella (HPS)

Serotype / size (bp)	n	HPS-6	HPS-1	HPS-3	HPS-5	HPS-4	HPS-2	invA	number of targets detected
Typhimurium	171	99.4	28.1	100.0	100.0	69.6	100.0	100.0	5-7
Dublin	44	4.5	100.0	100.0	95.5	88.6	100.0	100.0	5-6
Enteritidis (80%)	24	0.0	8.3	100.0	100.0	100.0	100.0	100.0	5-6
Newport (93%)	172	100.0	2.3	100.0	100.0	0.0	99.4	100.0	5-6
1,4,[5],12:i:-	14	100.0	0.0	100.0	100.0	0.0	100.0	100.0	5-6
Heidleberg	22	100.0	4.5	100.0	100.0	0.0	0.0	100.0	4
Newport* (7%)	12	100.0	0.0	83.3	100.0	0.0	16.7	100.0	3-4
Lubbock	65	100.0	0.0	98.5	100.0	0.0	0.0	100.0	3-4
Mbandaka	39	97.4	0.0	100.0	100.0	0.0	0.0	100.0	3-4
Infantis	19	78.9	0.0	94.7	94.7	0.0	15.8	100.0	3-4
Muenchen	12	91.7	0.0	91.7	91.7	0.0	0.0	100.0	3-4
Thompson	11	9.1	9.1	90.9	90.9	0.0	54.5	100.0	3-4
Kentucky	56	60.7	0.0	96.4	94.6	0.0	0.0	100.0	3-4
Enteritidis* (20%)	6	0.0	0.0	66.7	83.3	0.0	83.3	100.0	3-4
Meleagridis	32	0.0	0.0	100.0	100.0	0.0	0.0	100.0	3
Agona	28	0.0	0.0	100.0	100.0	0.0	0.0	100.0	3
Anatum	288	100.0	0.0	100.0	0.0	0.0	0.0	100.0	3
Lille	46	100.0	0.0	0.0	100.0	0.0	0.0	100.0	3
Reading	16	87.5	0.0	12.5	75.0	0.0	12.5	100.0	3-4
Montevideo* (4.5%) Clade IV	7	28.6	14.3	100.0	28.6	0.0	14.3	100.0	3-4
Cerro	205	51.7	0.0	100.0	21.0	0.0	0.0	100.0	2-3
Muenster	59	6.8	0.0	6.8	0.0	0.0	10.2	100.0	2-3
Montevideo (95.5%) Clade I	148	0.0	0.0	0.0	0.0	0.0	0.0	100.0	1
EB (39); GB Enrichments (373)	412	0.0	0.0	0.0	1.0	0.0	0.0	0.0	0
Total samples tested:	1908								

DENT (bracket labeling the first five serotype rows: Typhimurium, Dublin, Enteritidis (80%), Newport (93%), 1,4,[5],12:i:-)

Table 1. *Salmonella* resulting in 5 or more bands are considered HPS. DENT: Dublin, Enteritidis, Newport, Typhimurium. *Salmonella* with 4 bands are not designated as HPS but have been attributed to outbreaks and can be found on the CDC's list of top 20 *Salmonella* causing illness in the US. *Salmonella* with ≤ 3 bands are designated as non-HPS and are predicted to be less pathogenic to humans.

To date, the HPS assay has been tested with over 1600 *Salmonella* isolates encompassing 78 serotypes. Preliminary validation testing using *Enterobacteriaceae* of various genera (n=39) has shown no cross reactivity for the assay. The assay was found to have 99% specificity and 96% sensitivity for identifying serotypes Dublin, Enteritidis, Newport and Typhimurium and use of the assay to identify *Salmonella* in > 400 ground beef enrichments showed no cross reactivity.

Salmonellosis results from the interplay of a number of factors including host immune status, infectious dose and *Salmonella* pathogenicity level. As such, mitigation strategies for *Salmonella* contamination in foods should include a quantitative assessment of both contamination level and pathogenicity level (Figure 1). The USDA-ARS is seeking an industry partner to help develop the HPS assay for application in the meat industry, to help identify *Salmonella* pathogenicity level. The ideal CRADA partner would have expertise in developing and marketing a rapid test for detecting pathogens in food and an interest in supporting the project both intellectually and financially.

Figure 1. *Salmonella* risk mitigation strategy.
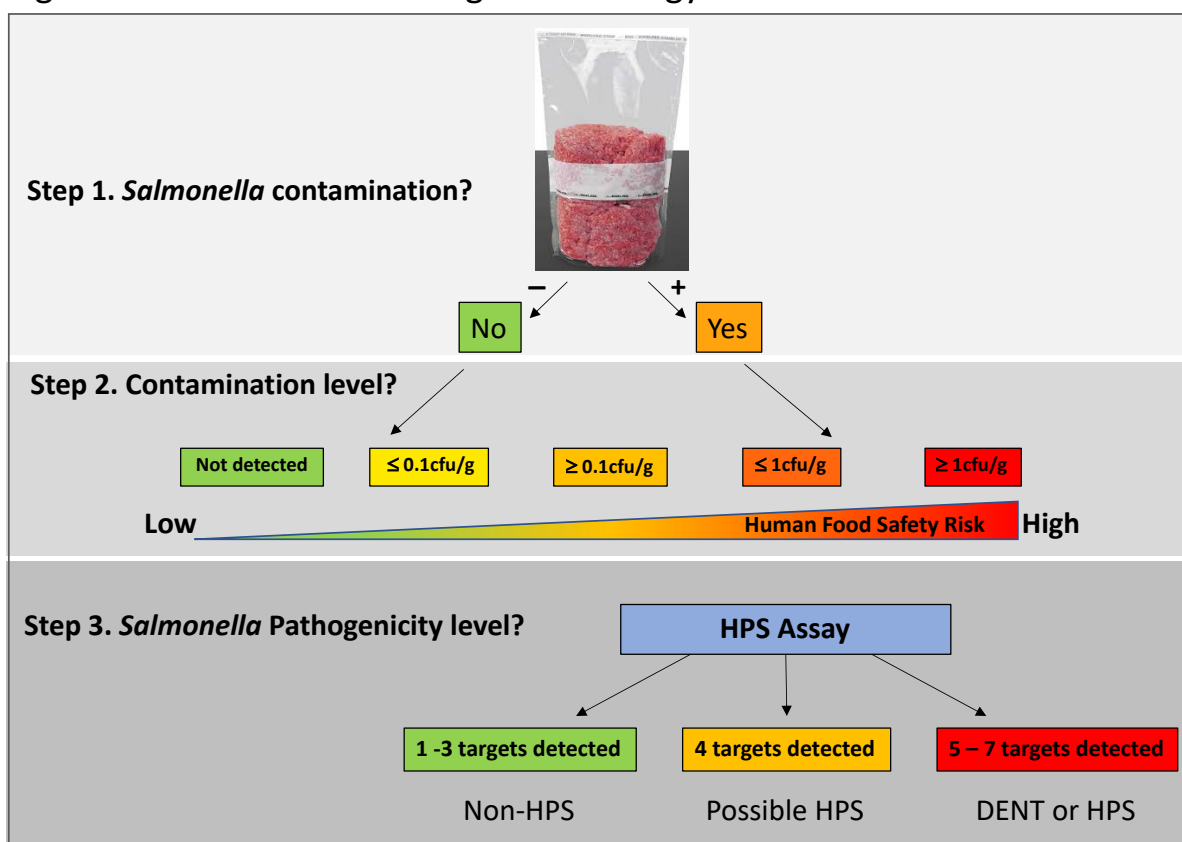
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